Remarks

Applicants have received and reviewed the Office Action mailed October 10, 2002. By way of response, Applicants have amended claims 1, 4-9 and 17-42 and present new claims 43-92. Claims 1, 4-10 and 17-92 are now pending. No new matter is introduced. Applicants submit that the amended and newly presented claims are supported by the specification. Applicants note that a declaration from Dr. P. Schlievert, referenced in the remarks below, will be submitted separately.

For the reasons given below, Applicants respectfully submit that the amended and newly presented claims are in condition for allowance, and notification to that effect is earnestly solicited.

97.5% & 99% Identity – Basis in the Specification

Claims 43-92 are now written to recite a percentage of identity with Streptococcal pyrogenic exotoxin type C (SPE-C). Basis for such claims exists in the specification and that no new matter is being added thereby. The Examiner's attention is directed to examples of the specification discussing up to 6 amino acid changes being made at pages 8 and 22 of the specification. Further, it is noted that the specification discloses that SPE-C is 235 amino acids in length at page 24 of the specification and also in Figure 1. Accordingly, when one calculates percentage identity using 229 amino acids that are identical (235-6) one arrives at a value of 97.45% identity, which is reasonably rounded off to 97.5%.

Examiner's attention is further drawn to pages 8 and 9 of the specification where it is disclosed that mutated toxins of the present invention preferably have up to 99% homology with wild-type SPE-C toxin.

Accordingly, there is support in the specification for claim language relating to both 97.5% identity and 99% identity with SPE-C.

35 U.S.C. § 112, Enablement

The Examiner rejected claims 1, 4-10, 17-19, 27-28 and 32-42 under 35 U.S.C. § 112, first paragraph. Applicants respectfully traverse this rejection. Although this rejection has not been raised for the newly presented claims, it is discussed insofar as it might apply.

Applicants maintain that the facts, authority, and reasoning presented in their submissions mailed February 26, 2001 and September 17, 2001 address the Examiner's comments regarding claims 1, 4-10, 17-19, 27-28 and 32-42. However, Applicants will address each point again so that the reason the claims should be allowed is clear.

The Office Action asserts the specification does not reasonably provide enablement for a Streptococcal pyrogenic exotoxin type C (SPE-C) mutant with three, four or five mutations at the recited positions. The Office Action goes on to reiterate this position by asserting that there are no examples that teach three, four or five substitutions at each of the recited positions, as well as making other points with respect to mutant polypeptides with three or more substituted positions.

However, the specification clearly enables mutant toxins with three or more substituted positions because it discloses both how such a mutant would be made as well as the specific locations at which the substitutions should occur. For example, the specification discloses a method of making mutants having more than two substations for example at p. 38, example 5. Further, the specification discusses locations for mutations by describing a mutant comprising an amino acid substitution in a β-barrel of a B-subunit or a N-terminal alpha helix. At least page 12, lines 8 - 17 of the specification supports mutations on β-barrel 4 of B-subunit 5. Particular amino acids supported as points for mutation in the β-barrels include His-35, Asn-38, Thr-33 and Leu-36. At least page 14, lines 14 - 22 of the specification supports mutations on N-terminal alpha helix 51. Particular amino acids supported as points for mutation in the N-terminal alpha helix include Ser-11, Asp-12, Tyr-15 and Tyr-17. At least page 14, line 22 through page 15, line 4 supports mutations on a central alpha helix. Particular amino acids supported as points for mutation in the central alpha helix include Lys-135, Lys-138, Tyr 139, and Asp-142. Thus, with a method of making mutant toxins with three or more substituted positions and with a disclosure that describes which specific locations should be substituted, the specification is enabling for one of skill in the art to produce mutant toxins with three or more substituted positions.

Further evidence of this enablement is provided by the declaration of Dr. P. Schlievert which shows that the teachings of the specification can be used to create mutant toxins with three or more substituted positions. Specifically, Dr. Schlievert has constructed triple mutants, including Y15A/H35A/N38D in accordance with the methods of the present invention that have reduced toxicity but adequate immunogenicity.

The Office Action asserts that if each recited position is substituted with any amino acid and in any combination of substitutions there would be an infinite number of possible combinations. However, Applicants submit that the number of possible combinations included within the scope of the present claims is definite and finite. The claims contemplate amino acid substitution at specific locations of SEQ ID NO: 2. Thus, the number of possible combinations is limited by both the range of amino acids that may be substituted into the isolated polypeptide and by the number of different positions in the isolated polypeptide of SEQ ID NO: 2 for which substitution is being carried out. With regard to the range of amino acids that may be substituted it is disclosed, starting at p. 10, line 24, that the substitutions may be made with the other 19 naturally occurring amino acids or with non-naturally occurring amino acids or analogs some of which are discussed starting at p. 11, line 7. With regard to the number of different positions in the isolated polypeptide of SEQ ID NO: 2, the claims specifically refer to sites on SEQ ID NO: 2 that may be substituted. Accordingly, there is not an infinite number of combinations claimed but rather a finite number of combinations.

Applicants further note that the amended and newly presented claims relating to mutants of SPE-C including particular substitutions at particular amino acids is fully supported. Each of these residues are specifically called out in the present specification as preferred locations for substitutions. For example, the specification discusses a mutant comprising an amino acid substitution in a β-barrel of a B-subunit or a N-terminal alpha helix. At least page 12, lines 8 - 17 of the specification supports mutations on β-barrel 4 of B-subunit 5. Particular amino acids supported as points for mutation in the β-barrels include His-35, Asn-38, Thr-33 and Leu-36. At least page 14, lines 14 - 22 of the specification supports mutations on N-terminal alpha helix 51. Particular amino acids supported as points for mutation in the N-terminal alpha helix include Ser-11, Asp-12, Tyr-15 and Tyr-17. At least page 14, line 22 through page 15, line 4 supports mutations on a central alpha helix. Particular amino acids supported as points for mutation in the central alpha helix include Lys-135, Lys-138, Tyr 139, and Asp-142.

The Office Action asserts that there are no examples using histidine-35 in combination with other amino acid substitutions. In response, applicants note that an example of using histidine-35 as a point of substitution was provided in the specification at p. 44. Applicants further note that examples were provided where single point mutations were then tested as double point mutations with success. An example of this is how the teachings of the Y15S single

mutant, at p. 39, and N38S single mutant, at p. 39, were then applied in the Y15S/N38S double mutant, also at p. 39. Thus, Applicants have demonstrated a reasonable expectation of success in creating multiple point mutants where the individual single point mutants have been successfully demonstrated.

To view this point otherwise would be requiring that the claims be limited to only the exemplified embodiments. However, authority found in the MPEP and the case law, which applicant has pointed out in the parent application and which should be well known to the Examiner, indicate that it is inappropriate to limit an invention to exemplified embodiments, particularly when the application provides factual support for broader claims.

The MPEP addresses the severity of limiting claims to exemplified embodiments:

In In re Goffe, 542 F.2d 564, 567, 191 USPQ 429, 431 (CCPA 1976), the court stated:

> [T]o provide effective incentives, claims must adequately protect inventors. To demand that the first to disclose shall limit his claims to what he has found will work or to materials which meet the guidelines specified for "preferred" materials in a process such as the one herein involved would not serve the constitutional purpose of promoting progress in the useful arts.

M.P.E.P. at 2164.08.

The MPEP notes that limiting an inventor to claims to preferred materials or what the inventor has found will work does not serve the constitutional purpose of promoting progress in the useful arts. In the present case, Applicants have exemplified numerous nonlethal mutants of SPE-C, have explicitly described various amino acids that are preferred sites for making such mutants, and specifically describe secondary structural features that are suitable locations for mutations eliminating toxicity. By the standard expressed in In re Goffe and in the MPEP at 2164.08, constitutional purposes would be defeated by limiting the inventor to the specifically disclosed mutants of SPE-C.

The Office Action asserts that specification only provides guidance to specific amino acids and does not teach any amino acid substitution may be changed without causing a detrimental effect to the SPE-C toxin to be produced. The Office Action further asserts that the claims do not recite whether the substitution will be a conservative substitution and expresses concern over producing a stable SPE-C toxin. The issues of detrimental effect, conservative substitutions, and protein stability are all interrelated and will therefore be addressed together.

The Applicants respectfully disagree with the Examiner regarding the relevancy of detrimental effect, conservative substitutions, and protein stability for this invention. An aspect of the mutant toxins of the present invention is their ability to be immunogenic. However, detrimental effect, conservative substitutions, and protein stability are simply irrelevant. The protein does not have to remain intact to function as intended. Substitutions do not have to be conservative for the mutants to function as immunogens. There is support for claims that the mutants can be immunogenic. Four double mutants (Y15A/N38A, Y17A/N38A, Y15S/N38S, and Y17S/N38S) were prepared as described in Example 5 and then evaluated in Example 6. The mutations were effective immunogens. Finally, there are no claims in the present invention regarding the stability of the mutations. Therefore, it is believed that questioning the detrimental effect, conservative substitutions, and protein stability of the mutants is inappropriate.

Conclusion

Accordingly, it is submitted that the amended and newly presented claims fully comply with § 112, first paragraph, and withdrawal of this rejection is respectfully requested.

35 U.S.C. § 112, Indefiniteness

The Examiner rejected claims 1, 4-10 and 17-42 under 35 U.S.C. § 112, second paragraph. The Office Action asserts the specification is indefinite for referring to amino acid substitutions without referring to a basic sequence which is being substituted. Though applicants traverse this rejection, claims 1, 4-9 and 17-42 have been amended, for the purpose of advancing prosecution, to include a sequence ID number rendering this rejection moot for these claims. It is further believed that this rejection will not apply to new claims 43-92 because these claims also recite a sequence ID number.

Accordingly, it is submitted that the amended and newly presented claims fully comply with § 112, second paragraph, and withdrawal of this rejection is respectfully requested.

Summary

In summary, Applicants assert that each of claims 1, 4-10, and 17-42 are in condition for allowance, and notification of that effect is earnestly solicited.

The Examiner is invited to contact Applicants' undersigned representative at the telephone number provided below, if the Examiner believes that doing so will expedite prosecution of the application.

Respectfully submitted,

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MARKED-UP VERSION TO SHOW CHANGES MADE

In the Specification

Please change the paragraph beginning at Page 1, Line 6 to the following:

This application claims the benefit of U.S. Provisional Application Serial No. 60/091,864 and is a Continuation-In-Part of U.S. Patent Application Serial No. [__/___,___] 09/308,829, filed [May 25] July 14, 1999, which is based on U.S. PCT Chapter II National Stage Application PCT/US97/22125, filed December 5, 1997, which claims priority to U.S. Provisional Application Serial No. 60/033,251, filed December 6, 1996.

Please change the paragraph beginning at Page 6, Line 8 to the following:

Figure 1 shows the nucleotide sequence of *speC* (SEQ ID NO: 1). Numbering is in reference to the ATG start codon. Possible promoter (-10, -35) and Shine-Dalgarno (SD) sequences are noted. The deduced amino acid sequence (SEQ ID NO: 2) is given below the nucleotide sequence. An asterisk after residue 27 indicates the cleavage site between the signal peptide and mature protein. Overlined nucleotides 3' of the translation stop codon are palindromic sequences.

Please change the paragraph beginning at Page 7, Line 29 to the following:

Wild type SPE-C toxin is encoded by a gene speC. The wild type SPE-C toxin has a molecular weight of 24,000 Daltons as determined by SDS PAGE of purified protein. A DNA sequence (SEQ ID NO: 1) encoding a wild type SPE-C toxin and the predicted amino acid sequence (SEQ ID NO: 2) for a wild type SPE-C toxin is shown in Figure 1. A DNA sequence encoding a wild type SPE-A toxin has been cloned in E. coli and S. aureus. Amino acid number designations in this application are made by reference to the sequence of Figure 1 with aspartate at position 28 designated as the first amino acid. The first 27 amino acids represent a leader sequence not present in the mature protein.

Please change the paragraph beginning at Page 8, Line 15 to the following:

As used herein, the definition of a wild type SPE-C toxin includes variants, such as allelic variants, of a wild type SPE-C toxin that have the same biological activity of wild type SPE-C

toxin. These SPE-C toxins may have a different amino acid or their genes may have a different nucleotide sequence from that shown in Figure 1 but do not have different biological activities. Changes in amino acid sequence are phenotypically silent. Preferably, these toxin molecules have systemic lethality and enhance endotoxin shock to the same degree as wild type SPE-C toxin shown in Figure 1. Preferably these toxins have at least 60-99% homology with wild type SPE-C toxin amino acid sequence (SEQ ID NO: 2) as shown in Figure 1 as determined using the SS2 Alignment Algorithm as described by Altschul,S. F., Bull. Math. Bio. 48:603 (1986). Proteins that have these characteristics substantially correspond to a wild type SPE C.

Please change the paragraph beginning at Page 9, Line 24 to the following:

Changes in the amino acid sequence at a particular site can be randomly made or specific changes can be selected. Once a specific site is selected it is referred to by its amino acid number designation and by the amino acid found at that site in the wild type SPE-C (SEQ ID NO: 2) as shown in Figure 1. The amino acid number designations made in this application are by reference to the sequence in Figure 1 with the aspartate at position 28 being counted as the first amino acid. Equivalent amino acids corresponding to those identified at a particular site in proteins substantially corresponding to a wild type SPE-C toxin may have different amino acid numbers depending on the reference sequence or if they are fragments. Equivalent residues are also those found in homologous molecules that can be identified as equivalent to amino acids in proteins substantially corresponding to a wild type SPE-C toxin either by comparison of primary amino acid structure or by comparison to a modeled structure as shown in Figure 1 or by comparison to a known crystal structure of a homologous molecule. It is intended that the invention cover changes to equivalent amino acids at the same or similar locations regardless of their amino acid number designation.

Please change the paragraph beginning at Page 28, Line 9 to the following:

A mutant DNA sequence encoding a mutant SPE-C toxin that has at least one change in amino acid sequence can be formed by a variety of methods depending on the type of change selected. A DNA sequence encoding a protein substantially corresponding to wild type SPE-C toxin functions as template DNA used to generate DNA sequences encoding mutant SPE-C toxins. A DNA sequence encoding wild type SPE-C toxin is shown in Figure 1 (SEQ ID NO: 1).

In the Claims

Marked-Up Version of Claims:

1. (3 Times Amended) A mutant Streptococcal pyrogenic exotoxin type C (SPE-C toxin):

the mutant comprising an amino acid substitution at aspartic acid-12 of SEQ ID NO: 2, tyrosine-15 of SEQ ID NO: 2, tyrosine-17 of SEQ ID NO: 2, histidine-35 of SEQ ID NO: 2, asparagine-38 of SEQ ID NO: 2, or substitution at more than one of these amino acids.

4. (3 Times Amended) The mutant SPE-C toxin of claim 1, wherein the amino acid substitution comprises:

the substitution of aspartic acid-12 of SEQ ID NO: 2 to alanine, glutamic acid, asparagine, glutamine, lysine, arginine, serine, or threonine;

the substitution of tyrosine-15 of SEQ ID NO: 2 to phenylalanine, alanine, glycine, serine, or threonine;

the substitution of tyrosine-17 of SEQ ID NO: 2 to phenylalanine, alanine, glycine, glutamic acid, lysine, arginine, aspartic acid, serine, or threonine;

the substitution of histidine-35 of SEQ ID NO: 2 to phenylalanine, alanine, glycine, glutamic acid, lysine, arginine, aspartic acid, tyrosine, phenylalanine, serine, or threonine;

the substitution of asparagine-38 of SEQ ID NO: 2 to alanine, aspartic acid, glutamic acid, lysine or arginine; or

substitution at more than one of these amino acids.

5. (3 Times Amended) The mutant SPE-C toxin of claim 4, wherein the amino acid substitution comprises:

the substitution of aspartic acid-12 of SEQ ID NO: 2 to alanine,

the substitution of tyrosine-1 of SEQ ID NO: 2 to alanine,

the substitution of tyrosine-17 of SEQ ID NO: 2 to alanine,

the substitution of histidine-35 of SEQ ID NO: 2 to alanine,

the substitution of asparagine-38 of SEQ ID NO: 2 to aspartic acid; or

substitution at more than one of these amino acids.

6. (Twice Amended) The mutant SPE-C toxin of claim 1, wherein the amino acid substitution comprises substitution of tyrosine-15 of SEQ ID NO: 2 and asparagine-38 of SEQ ID NO: 2.

7. (Twice Amended) The mutant SPE-C toxin of claim 6, wherein the substitutions are tyrosine-15 of SEQ ID NO: 2 to alanine and asparagine-38 of SEQ ID NO: 2 to alanine.

8. (Twice Amended) The mutant SPE-C toxin of claim 1, wherein the amino acid substitution comprises substitution of tyrosine-17 of SEQ ID NO: 2 and asparagine-38 of SEQ ID NO: 2.

9. (Twice Amended) The mutant SPE-C toxin of claim 8, wherein the substitutions are tyrosine-17 of SEQ ID NO: 2 to alanine and asparagine-38 of SEQ ID NO: 2 to alanine.

17. (Twice Amended) The mutant SPE-C toxin of claim 1, wherein the amino acid substitution comprises:

the substitution of tyrosine-15 of SEQ ID NO: 2 to alanine or serine;

the substitution of tyrosine-17 of SEQ ID NO: 2 to alanine or serine;

the substitution of asparagine-38 of SEQ ID NO: 2 to serine or alanine;

the substitution of tyrosine-15 of SEQ ID NO: 2 to serine or alanine and of asparagine-38 of SEQ ID NO: 2 to serine or alanine;

the substitution of tyrosine-17 of SEQ ID NO: 2 to serine or alanine and of asparagine-38 of SEQ ID NO: 2 to serine or alanine;

the substitution of aspartic acid-12 of SEQ ID NO: 2 to alanine;

the substitution of asparagine-38 of SEQ ID NO: 2 to aspartic acid; or

the substitution of tyrosine-15 of SEQ ID NO: 2 to alanine, histidine-35 of SEQ ID NO: 2 to alanine and asparagine-38 of SEQ ID NO: 2 to aspartic acid.

18. (Amended) The mutant SPE-C toxin of claim 6, wherein the substitutions comprise tyrosine-15 of SEQ ID NO: 2 to alanine and asparagine-38 of SEQ ID NO: 2 to aspartic acid.

19. (Twice Amended) A mutant SPE-C toxin comprising acid substitutions at aspartic acid-12 of SEQ ID NO: 2, tyrosine-15 of SEQ ID NO: 2, tyrosine-17 of SEQ ID NO: 2, histidine-35 of SEQ ID NO: 2, or asparagine-38 of SEQ ID NO: 2.

20. (Amended) A mutant SPE-C toxin comprising amino acid substitution at aspartic acid-12 of SEQ ID NO: 2.

21. (Amended) The mutant SPE-C toxin of claim 20, comprising substitution of alanine for aspartic acid-12 of SEQ ID NO: 2.

22. (Amended) A mutant SPE-C toxin comprising amino acid substitution at asparagine-38 of SEQ ID NO: 2.

23. (Amended) The mutant SPE-C toxin of claim 22, comprising substitution of aspartic acid for asparagine-38 of SEQ ID NO: 2.

24. (Amended) A mutant SPE-C toxin comprising amino acid substitutions at tyrosine-15 of SEQ ID NO: 2 and at asparagine-38 of SEQ ID NO: 2.

25. (Amended) The mutant SPE-C toxin of claim 24, comprising substitutions of serine or alanine for tyrosine-15 of SEQ ID NO: 2 and aspartic acid for asparagine-38 of SEQ ID NO: 2.

26. (Amended) The mutant SPE-C toxin of claim 24, comprising substitutions of serine for tyrosine-15 of SEQ ID NO: 2 and serine for asparagine-38 of SEQ ID NO: 2.

27. (Amended) The mutant SPE-C toxin of claim 24, further comprising amino acid substitution at histidine-35 of SEQ ID NO: 2 .

28. (Amended) The mutant SPE-C toxin of claim 27, comprising substitutions of alanine for tyrosine-15 of SEQ ID NO: 2, alanine for histidine-35 of SEQ ID NO: 2, and aspartic acid for asparagine-38 of SEQ ID NO: 2.

29. (Amended) A mutant SPE-C toxin comprising amino acid substitutions at tyrosine-17 of SEQ ID NO: 2 and at asparagine-38 of SEQ ID NO: 2.

30. (Amended) The mutant SPE-C toxin of claim 29, comprising substitutions of serine or alanine for tyrosine-17 of SEQ ID NO: 2 and aspartic acid for asparagine-38 of SEQ ID NO: 2.

31. (Amended) The mutant SPE-C toxin of claim 29, comprising substitutions of serine for tyrosine-17 of SEQ ID NO: 2 and serine for asparagine-38 of SEQ ID NO: 2.

32. (Amended) A mutant SPE-C toxin comprising amino acid substitutions at tyrosine-15 of SEQ ID NO: 2, at histidine-35 of SEQ ID NO: 2, and at asparagine-38 of SEQ ID NO: 2.

33. (Amended) The mutant SPE-C toxin of claim 32, comprising substitutions of alanine for tyrosine-15 of SEQ ID NO: 2, alanine for histidine-35 of SEQ ID NO: 2, and aspartic acid for asparagine-38 of SEQ ID NO: 2.

34. (Amended) A mutant SPE-C toxin comprising amino acid substitutions at aspartic acid-12 of SEQ ID NO: 2, at tyrosine-15 of SEQ ID NO: 2, at tyrosine-17 of SEQ ID NO: 2, at histidine-35 of SEQ ID NO: 2, at asparagine-38 of SEQ ID NO: 2, or at up to three of these amino acids.

35. (Amended) The mutant SPE-C toxin of claim 34, comprising substitutions of serine or alanine for tyrosine-15 of SEQ ID NO: 2 and aspartic acid for asparagine-38 of SEQ ID NO: 2.

36. (Amended) The mutant SPE-C toxin of claim 34, comprising substitutions of serine or alanine for tyrosine-17 of SEQ ID NO: 2 and aspartic acid for asparagine-38 of SEQ ID NO: 2.

37. (Amended) The mutant SPE-C toxin of claim 34, comprising substitutions of serine for tyrosine-15 of SEQ ID NO: 2 and serine for asparagine-38 of SEQ ID NO: 2.

38. (Amended) The mutant SPE-C toxin of claim 34, comprising substitutions of serine for tyrosine-17 of SEQ ID NO: 2 and serine for asparagine-38 of SEQ ID NO: 2.

39. (Amended) The mutant SPE-C toxin of claim 34, comprising substitutions of alanine for tyrosine-15 of SEQ ID NO: 2.

40. (Amended) The mutant SPE-C toxin of claim 34, comprising substitutions of alanine for tyrosine-15 of SEQ ID NO: 2, alanine for histidine-35 of SEQ ID NO: 2, and aspartic acid for asparagine-38 of SEQ ID NO: 2.

41. (Amended) The mutant SPE-C toxin of claim 34, comprising substitutions of aspartic acid for asparagine-38 of SEQ ID NO: 2.

42. (Amended) The mutant SPE-C toxin of claim 34, comprising substitutions of alanine for aspartic acid-12 of SEQ ID NO: 2.

Claims 43-92 are new.